Ciência Animal Brasileira
Brazilian Animal Science

DOI: 10.1590/1809-6891v26e-80477E

e-ISSN 1809-6891
Animal science | Research article

Glycosaminoglycans and vitamin C *in ovo* and the feeding of broiler chickens influence bone and cartilaginous histology

Glicosaminoglicanos e vitamina C *in ovo* e na alimentação de frangos de corte influencia a histologia óssea e cartilaginosa

Arthur Zuanetti Curti[1], Elaine Talita Santos[2], Lizandra Amoroso[2], Julyana Machado da Silva Martins[3], Jean Kaique Valentim*[4], Sarah Sgavioli[5]

1 Universidade Brasil, Descalvado, São Paulo, Brazil
2 Universidade Estadual Paulista (UNESP), Jaboticabal, São Paulo, Brazil
3 Universidade do Estado de Minas Gerais (UEMG), Ituiutaba, Minas Gerais Brazil
4 Universidade Federal Rural do Rio de Janeiro (UFRRJ), Seropédica, Rio de Janeiro, Brazil
5 Pontifícia Universidade Católica do Paraná (PUCPR), Curitiba, Paraná, Brazil
*corresponding author: kaique.tim@hotmail.com

Received: September 12, 2024. Accepted: April 15, 2025. Published: May 23, 2025. Editor: Rondineli P. Barbero

Abstract: The objective of this study is to evaluate the effect of *in ovo* feeding and dietary supplementation with glycosaminoglycans (GAGs) and vitamin C, on the bone and cartilage histology of broiler chickens. A completely randomized factorial design (2 x 2) was used, with two treatments during incubation (non-injected eggs and eggs injected with 4 µg of additive/100 µL of water on the fourth day of incubation), and two treatments during rearing (diet without additive and diet with 0.74 g of additive/100 kg of feed). Each 100 g of *in ovo* additive contained 30 g of chondroitin sulfate, 30 g of glucosamine sulfate, and 5 g of vitamin C, while each 100 kg of feed contained 30 g of glucosamine sulfate, 24 g of chondroitin sulfate, and 20 g of vitamin C. On day 43 of rearing, the number of chondrocytes in the cartilage, osteocytes, and periosteal osteoblasts in the tibial diaphysis, and tibial diaphysis thickness were evaluated. An interaction ($P < 0.05$) between the factors was observed. Greater diaphysis thickness and osteoblast numbers were observed in broilers fed with 0.74 g of additive/kg without *in ovo* injection, as well as in those that received 4 µg of additive in-ovo, and no additive in the diet. On the other hand, the use of the *in ovo* and dietary additive resulted in a reduction in the number of osteocytes. It was concluded that *in ovo* feeding or dietary supplementation with vitamin C and chondroitin and glucosamine sulfates during broiler rearing benefited bone and cartilage development.

Key-words: ascorbic acid; chondroitin; feed additives; glucosamine.

Resumo: O objetivo deste estudo foi avaliar o efeito da nutrição *in ovo* e da suplementação dietética com glicosaminoglicanos (GAGs) e vitamina C na histologia óssea e cartilaginosa de frangos de corte. Utilizou-se um delineamento fatorial inteiramente casualizado (2 x 2), com dois tratamentos durante a incubação (ovos não injetados e ovos injetados com 4 µg de aditivo/100 µL de água no quarto dia de incubação) e dois tratamentos durante a criação (dieta sem aditivo e dieta com 0,74 g de aditivo/100 kg de ração). Cada 100 g de aditivo *in ovo* continha 30 g de sulfato de condroitina, 30 g de sulfato de glucosamina e 5 g de vitamina C, enquanto cada 100 kg de ração continha 30 g de sulfato de glucosamina, 24 g de sulfato de condroitina e 20 g de vitamina C. Aos 43 dias de criação, foram avaliados o número de condrócitos na cartilagem, o número de osteócitos e osteoblastos periosteais na diáfise da tíbia e a espessura da diáfise tibial. Houve

interação (P < 0,05) entre os fatores avaliados. As maiores espessuras de diáfise e número de osteoblastos foram observadas nos frangos alimentados com 0,74 g de aditivo/kg de ração e sem aditivo *in ovo*, assim como naqueles que receberam 4 µg de aditivo *in ovo* e não receberam aditivo na dieta. Por outro lado, o uso do aditivo *in ovo* e na dieta resultou em uma redução do número de osteócitos. Conclui-se que a nutrição *in ovo* ou a suplementação dietética com vitamina C e sulfatos de condroitina e glucosamina durante a criação de frangos de corte favorece o desenvolvimento ósseo e cartilaginoso.

Palavras-chave: ácido ascórbico; condroitina; alimentação com aditivos; glucosamina.

1. Introduction

The rapid growth and high rate of meat deposition in broilers are associated with various metabolic disorders, which often result in locomotor problems such as leg weakness, joint issues, and locomotion-related deformities [1,2]. Locomotor disorders affect many broiler chickens, particularly in the late growth phase [3] and in fast-growing lines [4]. These disorders are associated with higher mortality rates, lower slaughter weights, and increased carcass condemnations, resulting in significant economic losses (5,6). Moreover, locomotion deformities reduce the welfare of broiler chickens by increasing discomfort during walking and limiting their ability to exhibit normal behaviors [3,4,5].

Some nutraceuticals can prevent and/or reduce the progression of pathological changes in locomotor structures. Among these products, polysulfated glycosaminoglycans (GAGs), chondroitin, and glucosamine sulfates have been shown to benefit the development of bone and joint structures in broilers [7, 8, 9, 10], leading to a consequent reduction in locomotor problems [11] and improved performance, as evidenced by improved weight gain [12] and reduced feed conversion [7].

GAGs, such as chondroitin and glucosamine sulfates, stimulate anabolic cartilage processes such as proteoglycan and collagen synthesis [13], chondrocyte proliferation [7, 10], bone matrix biosynthesis [10], and reduced bone resorption [13]. Moreover, they prevent cartilage degeneration through anti-inflammatory and inhibitory mechanisms [14]. Through epigenetic mechanisms, there is an increased expression of tissue inhibitory genes and a consequent reduction in the expression of matrix metallopeptidase enzymes [10]. On the other hand, vitamin C, or ascorbic acid, is an important nutrient in bone and cartilage development. Although it is synthesized endogenously, dietary supplementation can still be beneficial. This may be due to the insufficient availability of plasma vitamin C caused by a reduced capacity for synthesis under heat stress [15].

This finding suggests that maintaining a balance between the demand for and availability of vitamin C during heat stress can have beneficial effects. Barrio *et al.* [16] found that vitamin C supplementation improved growth performance in heat-stressed broiler chickens. In addition to enhancing performance, especially under heat-stress conditions, vitamin C also strengthens the immune system when added to the diet of broiler chickens. Furthermore, the electron-transferring ability of vitamin C gives it exceptional antioxidant qualities, helping to maintain the integrity of various cells [17]. Finally, vitamin C plays a crucial role in the development of both bone [8,9] and cartilage [9] in broiler chickens.

Studies have shown that *in ovo* inoculation with additives may improve hatching conditions, as reflected by chick quality [18, 19]. Thus, it may improve the physiological and biochemical stages of embryo development, providing benefits to the poultry industry [19]. Considering the existing research concerning the benefits of vitamin C and/or chondroitin and glucosamine sulfate supplementation in broilers [7,8,9,10,11,12,18], and the lack of studies on the effects of these additives, particularly *in ovo*, on bone

and cartilage development, this study was carried out to evaluate the influence of the use of GAGs and vitamin C *in ovo* and in feed on the bone and cartilage histology of broiler chickens.

2. Material and methods

This study was conducted following the ethical principles for animal experimentation adopted by the Brazilian College of Animal Experimentation (COBEA), with experimental procedures approved by the local Ethics Committee for Animal Use under protocol no. 011424/13 at the College of Agricultural and Veterinary Sciences, São Paulo State University, Jaboticabal, São Paulo, Brazil. The experiment was carried out at the College of Agricultural and Veterinary Sciences, São Paulo State University, located at 21° 14' 05" S latitude and 48° 17' 09" W longitude, at an average altitude of 615 meters.

A completely randomized 2 × 2 factorial design was used, with two treatments during the incubation phase (non-injected eggs and eggs injected with 4 µg of additive/100 µL of water on day four of incubation) and two treatments during the rearing/growth phase (diet without additive inclusion and diet with 0.74 g of additive/100 kg of feed). A total of 2,016 fertile eggs from 50-week-old Cobb® 500 broiler hens, with an average weight of 67±1.16 g, were distributed into seven trays (replicates), with 72 eggs per treatment per tray (504 eggs per treatment).

Eggs were incubated in incubators (CASP®, IHM Line e-V A 06, Amparo, Sao Paulo, Brazil) equipped with automatic temperature, humidity and egg-turning control. The initial and final temperatures and humidity were 37.8 and 36.9°C and 85.7 and 84.0%, respectively, after 504 hours of incubation.

According to Shim & Pesti [20], in broiler embryos, the bone system starts to develop on the fourth day of incubation; for this reason, this date was chosen. Each 100 g of *in ovo* additive was injected with 30 g of chondroitin sulfate, 30 g of glucosamine, and 5 g of vitamin C (Synth, 99% purity; Diadema, Brazil) or vehicle (100 g). The chondroitin sulfate (C14H21NO14S)n, Infiniti Nutraceuticals, Inc.) had a purity of 91.35%, and the potassium glucosamine sulfate (C6H14NO5)2 SO4 × 2KCl, Zheijiang Freemen, Inc.) had a sulfate content of 15.7%.

The formulation of the additive injected *in ovo* was based on the veterinary medicine Condrodoton 500 µg (Vetnil®, Louveira, Sao Paulo, Brazil), which is recommended for dogs. The supplied quantities of chondroitin sulfate, glucosamine sulfate, and vitamin C in micrograms were thus converted according to the dog weight in pounds to the quantity in micrograms of those substances for broiler weight in pounds. The results of studies on broiler nutrition [21, 7] were also used to adjust the glucosamine, chondroitin, and vitamin C doses in the additive injected.

For the additive injection, the eggs were held horizontally, and after cleaning with 70% ethanol, the shell was perforated near the thin end (the end opposite to the air cell) with a sterile needle [Injex, 13 × 0.38 (27.5 G1/2")] through which 100 µL aqueous additive solution was injected into the albumen, approximately 6 mm below the membrane. The solution was prepared with ultrapure water autoclaved in the dark because of its photosensitivity [22]. Each inoculated egg received the following constituents for treatment with 4 µg of the additive: 1.2 µg of chondroitin sulfate + 1.2 µg of glucosamine sulfate + 0.2 µg of vitamin C. After injection, the well was sealed with a label identifying the treatment and replicate.

After hatching, the males with one-day-old chicks were housed in 40 pens with 19 birds each, resulting in 10 replicates per treatment, totaling 760 birds. The chicks were reared until 42 days of age. The chicks received feed and water ad libitum throughout the experimental period and were raised

following the lighting, temperature, humidity, and management recommendations of the Cobb-Vantress Management Guide (23). The chicks were vaccinated in the hatchery against Marek's disease, infectious bursal disease (IBD), and avian pox. The following vaccination program was completed during the experimental period: IBD (mild strain) on the 7th day using eye drops; Newcastle disease and IBD (hot strain) through the drinking water; and the use of powdered milk as a carrier (2 g L-1) on day 14. The broilers were raised on wood-shaving litter with 1.2 kg of dry substrate per bird. The light regimen used was 24 L:0 D (light: dark).

The temperature and relative humidity were recorded daily via two digital thermohyrometers (Instrutemp, ITHT-2250, temperature scale from -50°C to 70°C, precision: ±1°C, São Paulo, Brazil), which were placed at the birds' height in two equidistant places. The average and absolute maximum temperatures were 30°C and 34.1°C, respectively, while the average and absolute minimum temperatures were 18.6°C and 13.4°C, respectively. The average and absolute maximum relative humidities were 73.36% and 36.18%, respectively, and the average and absolute minimum relative humidities were 27.82% and 15.00%, respectively.

The diets were based on corn and soybean meal (Table 1) and formulated in two phases: starter (1-21 days of age) and grower (22-42 days of age), as recommended by ROSTAGNO *et al*. [(24)]. For each 100 kg of feed, 30 g of glucosamine sulfate, 24 g of chondroitin sulfate, and 20 g of vitamin C were used.

Table 1. Ingredients and nutritional composition of diets for the starter phase (1--21 days of age) and grower-finisher phase (21--42 days of age).

Ingredients (%)	Starter 1-21 days of age	Grower-finisher 22-42 days of age
Corn	51.97	59.72
Soybean meal 45%	39.13	31.19
Soybean oil	4.82	5.63
Dicalcium phosphate	1.95	1.29
Limestone particle	0.79	0.87
Salt (NaCl)	0.50	0.50
Vitamin and mineral supplements*	0.40	0.40
DL-Methionine 99%	0.32	0.28
L- Lysine HCl 78,5%	0.13	0.12
Total	100	100
Nutritional composition		
Metabolizable energy (kcal/kg)	3.050	3.200
Crude protein (%)	22.0	19.0
Calcium (%)	0.90	0.75
Available phosphorus (%)	0.47	0.34
Sodium (%)	0.21	0.21
Digestible methionine+cysteine (%)	0.90	0.80
Digestible methionine (%)	0.59	0.53
Digestible lysine (%)	1.20	1.00

*Nutrients per kilogram of diet: From 1-21 days of age - Vit. A 7,000 U.I., Vit. D3 3,000 U.I., Vit. E 25 U.I., Vit. K 0.98 mg, Vit. B1 (1.78 mg), Vit. B2 (9.6 mg), Vit. B6 (3.5 mg), Vit. B12 (10 µg), folic acid (0.57 mg), biotin (0.16 mg), niacin (34.5 mg), calcium pantothenate (9.8 mg), copper (0.12 g), cobalt (0.02 mg), iodine (1.3 mg), iron (0.05 g), manganese (0.07 g), zinc (0.09 mg), organic zinc (6.75 mg), selenium (0.27 mg), choline (0.4 g), growth promoter (zinc bacitracin) (30 mg), narasin+nicarbazin (0.1 g), and methionine (1.68 g). From 21--42 days of age, Vit. A 7,000 U.I., Vit. D3 3,000 U.I., Vit. E 25 U.I., Vit. K 0.98 mg, Vit. B1 (1.78 mg), Vit. B2 (9.6 mg), Vit. B6 (3.5 mg), Vit. B12 (10 µg), folic acid (0.57 mg), biotin (0.16 mg), niacin (34.5 mg), calcium pantothenate (9.8 mg), copper (0.12 g), cobalt (0.02 mg), iodine (1.3 mg), iron (0.05 g), manganese (0.07 g), zinc (0.09 mg), organic zinc (6.75 mg), selenium (0.27 mg), choline (0.6 g), growth promoter (avilamycin) (7.5 mg), (monensin sodium) (0.1 g), and methionine (1.4 g. 1 For each 100 kg of feed, 30 g of glucosamine sulfate, 24 g of chondroitin sulfate and 20 g of vitamin C were used.

On the 43rd day of growth, 32 birds (with average body weights close to the experimental unit's average body weight (±5%) were selected and identified with numbered leg bands. The selected birds were fasted for eight hours and then slaughtered by neck displacement, drained, plucked, and eviscerated. The right tibia and proximal epiphysis joint cartilage of each bird were removed and marked.

For the joint cartilage and bone histological evaluation, one cm from the proximal epiphysis cartilage and bone diaphysis of the right tibia were collected. The chondrocyte number in the cartilage, the number of osteocytes in the tibial diaphysis, the number of periosteal osteoblasts in the tibial diaphysis, and the thickness of the tibial diaphysis were measured. For thickness, cortical bone (external rigid layer covering the cancellous bone) was considered.

The joint cartilage and bones were fixed in a solution of formaldehyde (10%) for 24 h, decalcified in a solution of formic acid (5%) for 14 days at room temperature, washed in distilled water, and processed according to the usual methods for light microscopy. The samples were dehydrated in a series of increasing ethanol concentrations (70%, 80%, 90%, 95%, and 100%). Afterward, they were diaphanized in an ethanol-xylene solution (1:1), followed by xylene (100%, 3x), and embedded in a mixture of xylene–paraffin (1:1), followed by paraffin, which lasted approximately 45 min in each solution. Histologic semiserial cartilage and bone samples 6 µm thick were stained with hematoxylin-eosin.

Fort-eight slides were made, with twelve replications/treatments. Four semi-serial cuts were placed on each slide. Images were obtained via a system of registering and analyzing the images (Olympus BX50; CellSens, Tokyo, Japan). The thickness of the tibial diaphysis was measured 60 times/treatment/replication, with an objective lens ×4. For the number of osteocytes and osteoblasts, areas of 15.05 mm2 and 562.91 µm2, respectively, were used, and for the number of chondrocytes, an area of 869 mm2 was used [7], with objective lenses ×60, 60 and 10, respectively. Ten images per cut were obtained for a total of 40 images per treatment/replication.

The effects of incubation treatments (without and with 4 µg of additive *in ovo* feeding (IOF)) and rearing treatments (without and with 0.74 g/kg additive in feed nutrition (FN)) and their interactions (IOF × FN) on the variables studied were analyzed according to the experimental model: $Yijk = \mu + (IOF)i + (FN)J + (IOF \times FN)ij + eijk$. The data were checked for the presence of outliers and met the assumptions of normality of observational mistakes and homogeneity of variances. After issues that compromised these assumptions were not identified, the data were subjected to analysis of variance via the general linear model (GLM) procedure of SAS® [25]. The means differed significantly according to the F test at 5% probability.

3. Results

There was an interaction effect among the treatments on the thickness of the tibial diaphysis (P = 0.0267), the number of osteocytes in the tibial diaphysis (P = 0.0484) (Table 2). The greatest thickness of the tibial diaphysis was observed for broilers fed 0.74 g of additive/kg and no additive injected *in ovo* (P = 0.034) and for broilers that received 4 µg of additive *in ovo* and no additive in the diet (P = 0.0023) (Table 2).

In ovo, feeding with 4 µg of the additive and the inclusion of 0.74 g of additive/kg of feed in the broiler diet during rearing reduced (P <0.0001 and p = 0.0108, respectively) the number of osteocytes in the tibial diaphysis (Table 2). The addition of chondroitin glucosamine sulfates and vitamin C did not affect the number of chondrocytes in the cartilage (P > 0.05) (Table 2). The number of periosteal osteoblasts in the tibial diaphysis (P = 0.0203) (Figure 1). The greatest number of osteoblasts was observed for broilers fed 0.74 g of additive/kg and no additive injected *in ovo* (P = 0.0421) and for broilers receiving 4 µg of additive *in ovo* and no additive in the diet (P < 0.0001) (Figure 1).

Table 2. Effects of chondroitin and glucosamine sulfates and vitamin C *in ovo* feeding and feed nutrition on the thickness of the tibial diaphysis, the number of osteocytes in the tibial diaphysis, and the number of chondrocytes in the cartilage of broilers at 43 days of age.

	In ovo feeding (IOF)	Feed nutrition (FN)					Probability (P)		
		0 g/kg	0.74 [2] g/kg	P	Mean	SEM[3]	IOF	FN	IOF x FN
Thickness of diaphysis (µm)	Un injected egg	1,710.46	1,737.55a	0.8072	1,726.72	306.09	0.0593	0.0593	0.0267
	4 µg of additive [1]	1,737.54A		0.0027	1,589.17				
	P	0.8034	0.0034						
	Mean	1,727.39	1,580.93						
Osteocytes number (15.05 mm^2)	Un injected egg	4.86	4.73a	0.6047	4.79	0.23	<.0001	0.0108	0.0484
	4 µg of additive [1]	4.41A	3.58Bb	0.0023	4.02				
	P	0.0863	<0.0001						
	Mean	4.62	4.19						
Chondrocytes number (869 mm^2)	Un injected egg	663.91	667.91	0.7944	665.91	9.84	0.7980	0.9348	0.7605
	4 µg of additive [1]	669.71	667.40	0.8678	668.56				
	P	0.6919	0.9724						
	Mean	667.10	667.63						

A-B; a-b: Means followed by different uppercase letters in lines or lowercase letters in columns differ significantly (P<0.05). 1 4 µg of the additive: 1.2 µg of chondroitin sulfate, 1.2 µg of glucosamine sulfate, and 0.2 µg of vitamin C. 2 For each 100 kg of feed, 30 g of glucosamine sulfate, 24 g of chondroitin sulfate and 20 g of vitamin C were used. 3SEM: mean standard error. SEM: Standard error of the mean. IOF: Effect of *in ovo* feeding. FN: Effect of feed nutrition. IOF x FN: Interaction between *in ovo* feeding and feed nutrition.

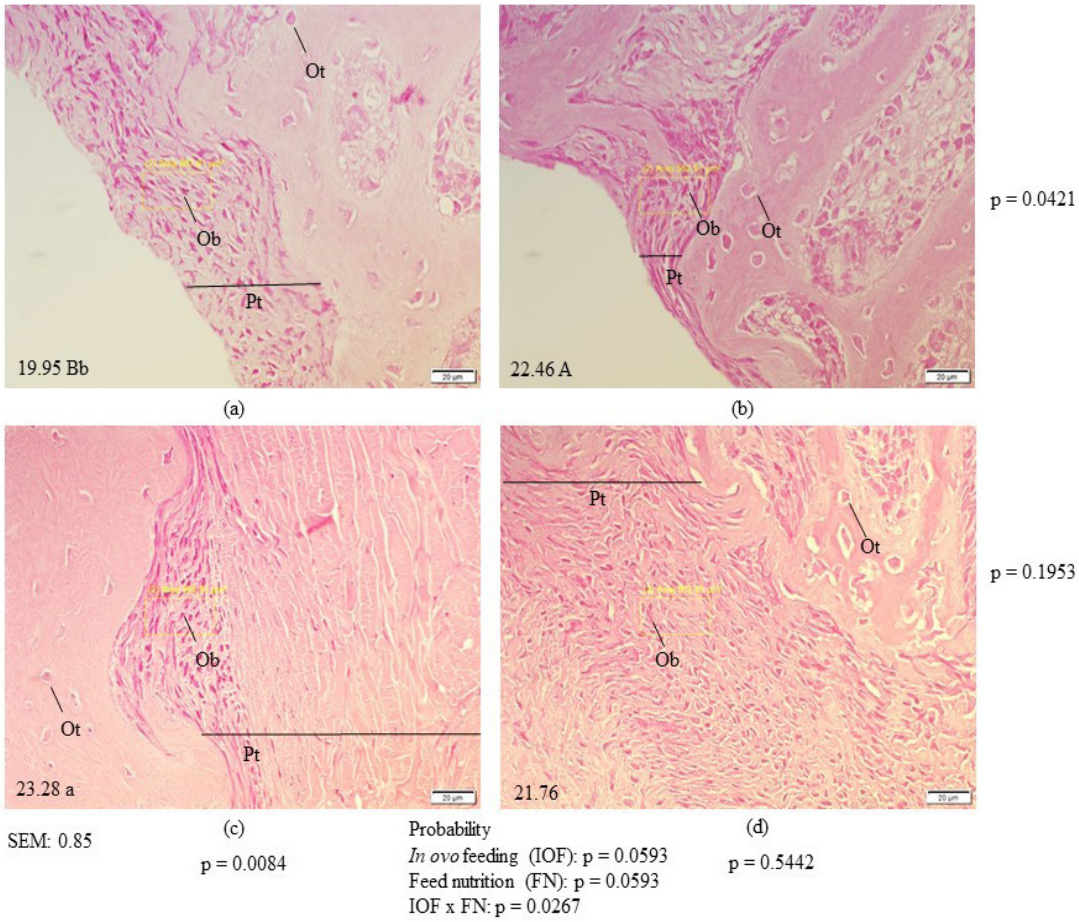

Figure 1. Effect of chondroitin and glucosamine sulfates and vitamin C *in ovo* feeding and feed nutrition on osteoblast number in the tibial diaphysis in broilers at 43 days of age. (a)-(d) Light micrographs of the tibial diaphysis bone of broilers. (a) *In ovo* feeding with 0 µg of the additive and the inclusion of 0 g of additive/kg of feed in the broiler diet during rearing; (b) ovo feeding with 0 µg of the additive and the inclusion of 0.74 g of additive/kg of feed in the broiler diet during rearing; (c) *in ovo* feeding with 4 µg of the additive and the inclusion of 0 g of additive/kg of feed in the broiler diet during rearing; (d) *in ovo* feeding with 4 µg of the additive and the inclusion of 0.74 g of additive/kg of feed in the broiler diet during rearing. Oc: osteocytes; Ob: osteoblasts; Pt: periosteum. SEM: mean standard error. Scale bar: 20 µm. A-B; a-b: means followed by different uppercase letters on *in ovo* feeding or lowercase letters on feed nutrition differ significantly ($p < 0.05$).

4. Discussion

In Ovo, feeding with chondroitin glucosamine sulfates and vitamin C was evaluated for its ability to promote histological changes in bone and cartilage development post-hatching. For the first time, this study revealed the effects of GAGs on the number of osteoblasts and the thickness of the diaphysis of broilers. According to the present study, the inclusion of GAGs and vitamin C in broiler feed efficiently promoted an increase in the thickness of diaphysis and the number of osteoblasts and osteocytes in the periosteum of the tibia diaphysis when used only *in ovo* or as a feed additive, which can benefit the development of broiler bone and cartilage, as verified by [9], and may represent a solution to bone problems in broilers.

Osteoblasts are the cells responsible for the synthesis and secretion of the constituents of the organic matrix of bone tissue [26]. They participate in the process of bone mineralization through the secretion of vesicles rich in alkaline phosphatase, which acts in the cleavage of pyrophosphate and removes its stabilizing influence. Moreover, they increase the local concentration of phosphate through their action on hexose monophosphate, a product derived from the breakdown of glycogen, promoting bone mineralization [26].

They indirectly participate in the bone resorption process by secreting cytokines, which stimulate osteoclasts [27]. Osteocytes are the most abundant cells in bone, and they are mature osteoblasts found within the bone matrix. They are responsible for maintaining the bone matrix, as they can synthesize and reabsorb the bone matrix according to the physiological needs of the animal but to a limited extent [28].

Therefore, the physiological role of osteoblasts, especially osteocytes, is important for the structural and biomechanical regulation of bone tissue mass [29]. An increase in these cells through exogenous supplementation with GAGs and vitamin C contributes to the inhibition of locomotor problems in broilers, which was previously verified by Martins *et al*. [12].

Santos *et al*. [9] also reported an increase in the number of osteocytes in the tibial diaphysis of broilers supplemented with chondroitin and glucosamine sulfates plus vitamin C. According to the authors, chondroitin and glucosamine sulfates probably stimulated the ossification of endochondral tissue, which led to an increase in the number of osteocytes in the growth plate. Concerning endochondral ossification, WOLFF [30] reported that the combination of glucosamine sulfate with chondroitin sulfate led to an increase in cell proliferation in ovariectomized rats, demonstrating that the growth plate of the tibial epiphysis promoted longitudinal bone growth after the administration of GAGs.

According to Choi *et al*. [31], vitamin C promotes osteoblast formation and blocks osteoclastogenesis through the activation of the wingless-type MMTV integration site family/β-catenin/activating transcription factor 4 signaling, which is achieved through the serine/threonine kinase and mitogen-activated protein kinase signaling pathways. According to Tat *et al*. [32], GAGs are compounds that modulate the expression of osteoprotegerin (OPG) and the activating receptor of the nuclear factor kappa-B ligand (RANKL), the two main factors involved in the bone remodeling process. Increasing OPG and decreasing RANKL gene expression increases the OPG/RANKL mRNA ratio, with a protective effect on bone loss. Therefore, a greater number of osteoblasts and greater thickness of the diaphysis can significantly contribute to the formation of long bones in broilers, thus favoring the locomotion of birds, as was observed for the first time in the literature [7].

This improvement in the development of the tibia may have been promoted by the addition of the product, leading to better locomotion, as found by Martins *et al*. [12], who studied the use of chondroitin and glucosamine sulfates in the prevention of locomotion problems in broilers and reported a reduction in the frequency of chickens with femoral degeneration, tibial dyschondroplasia and valgus and valgus deviations, in addition to improvements in the gait score.

5. Conclusion

In ovo feeding or dietary supplementation during the rearing of broilers with glycosaminoglycans (chondroitin sulfate and glucosamine sulfate) and vitamin C benefits bone and cartilage development.

Declaration of conflict of interest

The authors declare that they have no conflict of interest.

Data availability statement

The data will be provided upon request.

Author contributions

Conceptualization: Julyana Machado da Silva Martins, Elaine Talita Santos, and Lizandra Amoroso. Data curation: Elaine Talita Santos, Sarah Sgavioli, and Jean Kaique Valentim. Formal analysis: Arthur Zuanetti Curti and Sarah Sgavioli. Funding acquisition: Lizandra Amoroso, Julyana Machado da Silva Martins, and Elaine Talita Santos. Investigation: Arthur Zuanetti Curti. Methodology: Elaine Talita Santos and Lizandra Amoroso. Project administration: Sarah Sgavioli. Supervision: Elaine Talita Santos and Sarah Sgavioli. Writing (original draft): Arthur Zuanetti Curti, Elaine Talita Santos, and Sarah Sgavioli. Writing (review and editing): Sarah Sgavioli, Lizandra Amoroso, and Jean Kaique Valentim.

References

1. Guevara-Torres BL, Landin-Grandvallet LA, Tirado-Madrid A, Villagómez-Cortés JA. Comparison of locomotion problems and its economic impact on Cobb and Ross broiler strains. Veterinary Science Research. 2021; 3:1–6. DOI: https://journals.bilpubgroup.com/index.php/vsr/article/view/4126

2. Santos MN, Widowski TM, Kiarie EG, Guerin MT, Edwards AM, Torrey S. In pursuit of a better broiler: walking ability and incidence of contact dermatitis in conventional and slower growing strains of broiler chickens. Poultry Science. 2022; 101:1-19. DOI: https://doi.org/10.1016/j.psj.2022.101768

3. Wilcox CH, Sandilands V, Mayasari N, Asmara IY, Anang A. A literature review of broiler chicken welfare, husbandry, and assessment. World's Poultry Science Journal. 2024; 80:3–32. DOI: http://dx.doi.org/10.1080/00439339.2023.2264824

4. Nicol CJ, Abeyesinghe SM, Chang YM. An analysis of the welfare of fast-growing and slower-growing strains of broiler chicken. Frontiers in Animal Science. 2024; 5:1-21. DOI: https://doi.org/10.3389/fanim.2024.1374609

5. Granquist EG, Vasdal G, De Jong IC, Moe RO. Lameness and its relationship with health and production measures in broiler chickens. Animal. 2019; 13:2365–2372. DOI: https://doi.org/10.1017/s1751731119000466

6. Yang X, Zhao Y, Gan H, Hawkins S, Eckelkamp L, Prado M, Tabler T. Modeling gait score of broiler chicken via production and behavioral data. Animal. 2023; 17:1-10. DOI: https://doi.org/10.1016/j.animal.2022.100692

7. Sgavioli S, Santos ET, Borges LL, Andrade-Garcia GM, Castiblanco DM, Almeida VR, Baraldi-Artoni SM. Effect of the addition of glycosaminoglycans on bone and cartilaginous development of broiler chickens. Poultry Science. 2017; 96:4017–4025. DOI: https://doi.org/10.3382/ps/pex228

8. Santos ET, Sgavioli S, Castiblanco DMC, Domingues CHDF, Quadros TCOD, Borges LL, Baraldi-Artoni SM. Glycosaminoglycans and vitamin C *in ovo* feeding affects bone characteristics of chicks. Revista Brasileira de Zootecnia. 2018; 47:1-6. DOI: https://doi.org/10.1590/rbz4720170304

9. Santos ET, Sgavioli S, Castiblanco DM, Borges LL, de Quadros TC, de Laurentiz AC, Baraldi-Artoni SM. Glycosaminoglycans and vitamin C affect broiler bone parameters. Poultry Science. 2019; 98:4694–4704. DOI: https://doi.org/10.3382/ps/pez177

10. Martins JM, dos Santos Neto LD, Sgavioli S, Araújo IC, Reis AA, Santos RS, Café MB. Effect of glycosaminoglycans on the structure and composition of articular cartilage and bone of broilers. Poultry Science. 2023; 102:1-7. DOI: https://doi.org/10.1016/j.psj.2023.102916

11. Martins JMS, Santos Neto LDD, Gomides LPS, Fernandes EDS, Sgavioli S, Stringhini JH, Café MB. Performance, nutrient digestibility, and intestinal histomorphometry of broilers fed diet supplemented with chondroitin and glucosamine sulfates. Revista Brasileira de Zootecnia. 2020; 49:1-10. DOI: https://doi.org/10.37496/rbz4920190248

12. Martins JM, dos Santos Neto LD, Noleto-Mendonça RA, de Carvalho GB, Sgavioli S, de Carvalho FB, Café MB. Dietary supplementation with glycosaminoglycans reduces locomotor problems in broiler chickens. Poultry Science. 2020; 99:6974–6982. DOI: https://doi.org/10.1016/j.psj.2020.09.061

13. Maxwell LK, Regier P, Achanta S. Comparison of glucosamine absorption after administration of oral liquid, chewable, and tablet formulations to dogs. Journal of the American Animal Hospital Association. 2016; 52:90–94. DOI: https://doi.org/10.5326/jaaha-ms-6267

14. Kantor ED, Lampe JW, Navarro SL, Song X, Milne GL, White E. Associations between glucosamine and chondroitin supplement use and biomarkers of systemic inflammation. The Journal of Alternative and Complementary Medicine. 2014; 20:479–485. DOI: https://doi.org/10.1089/acm.2013.0323

15. Goel A. Heat stress management in poultry. Journal of Animal Physiology and Animal Nutrition. 2021; 105:1136–1145. DOI: https://doi.org/10.1111/jpn.13496

16. Del Barrio AS, Mansilla WD, Navarro-Villa A, Mica JH, Smeets JH, Den Hartog LA, García-Ruiz AI. Effect of mineral and vitamin C mix on growth performance and blood corticosterone concentrations in heat-stressed broilers. Journal of Applied Poultry Research. 2020; 29:23–33. DOI: https://doi.org/10.1016/j.japr.2019.11.001

17. Van Hieu T, Guntoro B, Qui NH, Quyen NTK, Al Hafiz FA. The application of ascorbic acid as a therapeutic feed additive to boost immunity and antioxidant activity of poultry in heat stress environment. Veterinary World. 2022; 15:685-693. DOI: https://doi.org/10.14202/vetworld.2022.685-693

18. Araújo IC, Café MB, Noleto RA, Martins JM, Ulhoa CJ, Guareshi GC, Leandro NS. ffect of vitamin E *in ovo* feeding to broiler embryos on hatchability, chick quality, oxidative state, and performance. Poultry Science. 2019; 98:3652–3661. DOI: https://doi.org/10.3382/ps/pey439

19. Peebles ED. *In ovo* applications in poultry: a review. Poultry Science. 2018; 97:2322–2338. DOI: https://doi.org/10.3382/ps/pey081

20. Shim MY, Pesti GM. Effects of incubation temperature on the bone development of broilers. The Journal of Poultry Science. 2011; 90:1867–1877. DOI: https://doi.org/10.3382/ps.2010-01242

21. Ferreira IB, Junior JM, Sgavioli S, Vicentini TI, Morita VS, Boleli IC. Vitamin C prevents the effects of high rearing temperatures on the quality of broiler thigh meat. Poultry Science. 2015; 94:841–851. DOI: https://doi.org/10.3382/ps/pev058

22. Sgavioli S, Borges LL, de Almeida VR, Thimotheo M, de Oliveira JA, Boleli IC. Egg injection of ascorbic acid stimulates leucocytosis and cell proliferation in the bursa of Fabricius. International Journal of Poultry Science. 2013; 12:464-472. DOI: http://dx.doi.org/10.3923/ijps.2013.464.472

23. Cobb-Vantress. Manual de manejo de frangos de corte. 2008. Disponível em: https://wp.ufpel.edu.br/avicultura/files/2012/04/Cobb-Manual-Frango-Corte-BR.pdf

24. Rostagno HS, Albino LFT, Donzele JL, Gomes PC, Oliveira RD, Lopes DC, Euclides RF. Composição de alimentos e critérios nutricionais. Tabelas brasileiras para aves e suínos, 2ª edição. UFV, Viçosa, MG, 2011.

25. SAS Institute. SAS/STAT user's guide: statistics. Version 9.2. Cary (NC): SAS Institute Inc.; 2002.

26. Pizauro Junior JM, Ciancaglini P, Macari M. Discondroplasia tibial: mecanismos de lesão e controle. Brazilian Journal of Poultry Science. 2002; 4:169–186. DOI: https://doi.org/10.1590/S1516-635X2002000300001

27. Duplomb L, Dagouassat M, Jourdon P, Heymann D. Concise review: embryonic stem cells: a new tool to study osteoblast and osteoclast differentiation. Stem Cells. 2007; 25:544–552. DOI: https://doi.org/10.1634/stemcells.2006-0395

28. Pizauro Júnior JM, Santos L, Gonçalves A. Estrutura e função do tecido ósseo. In: Fisiologia das aves comerciais. Jaboticabal: FUNEP; 2017.

29. Florencio-Silva R, Sasso GRDS, Sasso-Cerri E, Simões MJ, Cerri PS. Biology of bone tissue: structure, function, and factors that influence bone cells. BioMed Research International. 2015; 2015:1-17. DOI: https://doi.org/10.1155/2015/421746

30. Wolff RB. Glucosamine and chondroitin sulfate association increases tibial epiphyseal growth plate proliferation and bone formation in ovariectomized rats. Clinics. 2014; 69:847–853. DOI: https://doi.org/10.6061/clinics/2014(12)10

31. Choi HK, Kim GJ, Yoo HS, Song DH, Chung KH, Lee KJ, Koo YT, An JH. Vitamin C activates osteoblastogenesis and inhibits osteoclastogenesis via Wnt/β-Catenin/ATF4 signaling pathways. Nutrients. 2019; 11:1-20. DOI: https://doi.org/10.3390/nu11030506

32. Tat SK, Pelletier JP, Vergés J, Lajeunesse D, Montell E, Fahmi H, Martel-Pelletier J. Chondroitin and glucosamine sulfate in combination decrease the pro-resorptive properties of human osteoarthritis subchondral bone osteoblasts: a basic science study. Arthritis Research & Therapy. 2007; 9:1–10. DOI: https://doi.org/10.1186/ar2325